SYNTHESIS AND CHARACTERIZATION OF CADMIUM SULPHIDE NANOPARTICLES AND ITS UTILIZATION IN REMOVAL OF CADMIUM FROM AQUEOUS SOLUTION

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CERTIFICATE

This is to certify that the project report entitled **"Synthesis and characterization of cadmium sulphide nanoparticles and its utilization in removal of cadmium from aqueous solution"** submitted by **Ms. Kalpana Dalei** to the Department of Life Science, National Institute of Technology, Rourkela in partial fulfillment of the requirements for the degree of Masters of Science in **LIFE SCIENCE** is a bonafide record of work carried out by her under my supervision. The contents of this report in full or parts have not been submitted to any other Institute or University for the award of any degree or diploma.

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Kalpana Dalei

DECLARATION

I hereby declare that the project report entitled is submitted by ***Kalpana Dalei,*** **Roll no. 412ls2034** is an original work done and submitted by me in partial fulfilment for the Degree of Master of Science in Life Science , NIT Rourkela . This is a record work done by me under the guidance of ***Dr. Surajit Das,*** Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela.

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List of Abbreviations and Symbols

EPS	Extracellular Polymeric Substances
CdS	Cadmium Sulphide
FESEM	Field Emission Scanning Electron Microscope
XRD	X-Ray Diffraction
FTIR	Fourier Transformed Infra-red Spectroscopy
ATR	Attenuated Total Reflectance
µl	Microlitre
^{o}C	Degree Celsius
rpm	Revolution per Minute
hr	Hour
min	Minute
sec	Second
θ	Theta

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Abstract

Nanomaterials are the leading edge to the rapidly developing nanotechnology. For the synthesis of nanomaterials over a wide range of chemical composition, the development of reliable and efficient methods is a challenging issue in nanotechnology. In context to this green synthesis of nanoparticles using biological systems, plant products and microorganisms are potentially considerable because of its non-toxic and environmental friendly nature. The use of bacterial extracellular polymeric substances (EPS) is a current approach in the synthesis of nanoparticles. Cadmium sulphide (CdS) nanoparticles were synthesized by chemical method using glucose and biological method using EPS and surface functionalized EPS. EPS functionalization was carried out by attaching sulphur groups on its surface to synthesize CdS nanoparticles and simultaneous removal of cadmium from aqueous solution. The nanoparticles synthesized were characterized by UV-Visible spectrophotometer, XRD, ATR-FTIR and FESEM. The study also revealed that the percentage removal of cadmium from aqueous solution by the surface functionalized EPS was greater (80 %) than that of EPS (61%) which attributed to greater cadmium binding by sulphur group of the surface functionalized EPS. The percentage removal of cadmium also increased (83%) by using nanoparticle incorporated functionalized EPS, which provided a greater surface area for cadmium adsorption. Therefore, our work suggests that EPS functionalization is a feasible approach for CdS nanoparticles synthesis and removal of cadmium from aqueous solution.

Keywords: Nanoparticles, Marine bacteria, Extracellular polymeric substances, Cadmium sulphide, heavy metal, remediation.

Introduction

Nanoscience and nanotechnology have been an interesting field of research and gained much importance from last two decades. In layman terms "The study of properties of materials at nanoscale is termed as nanoscience, whereas the fabrication and application of the nanostructures are termed as nanotechnology". Nanoparticles are the smallest of the microscopic particles or ultrafine particles which is having size in the range of 1-100nm (Saxena *et al.* 2010). Nanoscale dimensions of these particles give them high surface area to volume ratio that contribute to enhanced chemical activity, high surface plasmon resonance (SPR), enhanced Rayleigh scattering and surface enhanced Raman Scattering than compared their bulk material . The applications of nanoparticles are various such as signal reporters to detect various biomolecules in immunoassay, as flurophore in fluorescence in situ hybridization (FISH) (Du 2005), cell bioimaging, an antioxidant to remove free radicals from patient blood stream, delivering vaccines and drugs (Gupta and Sharma 2011), treating infectious diseases, detection of cancer, fabrication of scaffolds and also more importantly in bioremediation (Fig. 1). Among various nanoparticles a great interest has been shown towards cadmium sulphide (CdS) nanoparticles because of availability of discrete energy levels, size dependent optical properties, tunable bandgap and a well-developed synthetic protocol, easy preparation technique with good chemical stability (Antolini *et al*. 2005). CdS nanoparticles categorised under the group chalcogenides and are an II-IV group semiconductor nanoparticle which shows size dependent optical and electrical properties due to its high surface area to volume ratio and quantum confinement (Bansal *et al*. 2012). Due to its very high photosensitivity it has usage in detection of visible radiations, in light emitting diodes, solar cells, photochemical catalysis, gas sensors, various luminescence devices, optoelectronic devices and a range of biological application (Chen *et al*. 1997). Semiconducting optoelectronic materials play an important role in a variety of application due to their unique optical, electrical, magnetic and piezoelectric properties. Modification of these properties of semiconductor materials depends upon the size, shape, morphology and dimensions of material (Hu *et al*. 1998). Due to this size dependent properties of semiconductors researchers interest turn towards the synthesis of nanometer range dimension which is comparable to Bohr radius. Such particles with Bohr radius may lead to quantum dot lasers, single electron transistors and several other biological applications (Ling *et al*. 1998). Cadmium sulphide (CdS)

semiconductor is an excellent visible light detector among other semiconductors (Ghasemi *et al*. 2009).

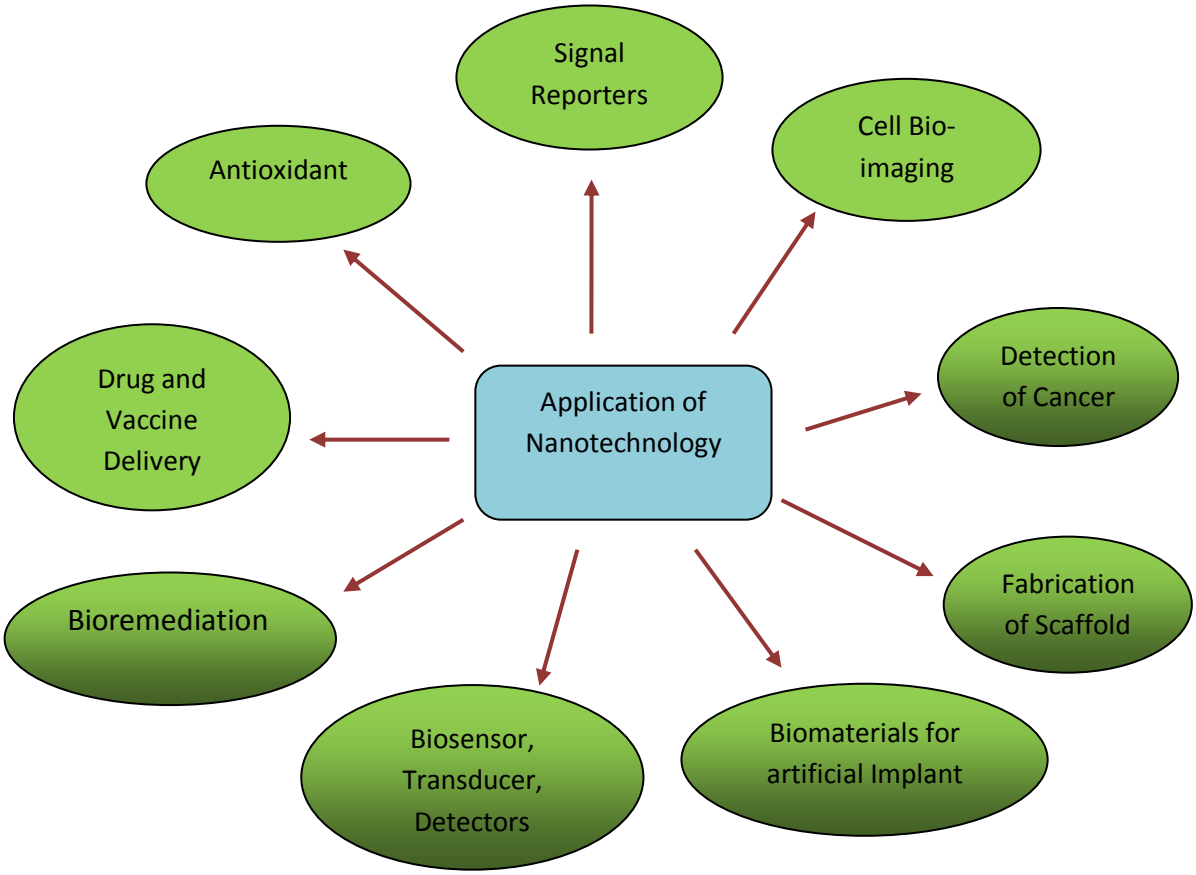


Fig.1. Different applications of nanotechnology

There are various physical, chemical and biological methods for synthesizing CdS nanoparticles (Fig. 2). In laboratory CdS nanoparticles can be synthesized by chemical methods and biological methods. Biosynthesis of CdS nanoparticles using various plant products and microorganisms had gained much more importance than the other methods as it is less expensive and more environment-friendly. Most of the chemical synthesis methods of CdS nanoparticles uses cadmium nitrate (Cd $(NO_3)_2$) and sodium sulphide (Na_2S) as precursors. In this case cadmium nitrate acts as a source of Cd^{+2} and sodium sulphide as source of S^{-2}.

A part from chemical methods, researchers are more focussing on the biological methods using various microorganisms for CdS synthesis which should be cost effective and environmental friendly. Such as biosynthesis of CdS nanoparticles is carried out by using

various microorganisms which can grab the Cd^{+2} ions from their metal solution and accumulate them in a reduced state by different enzymes and metabolites secreted from microbial activity (Mousavi *et al*. 2012). There are two different modes of synthesis of nanoparticles by microorganisms; intracellular and extracellular which is based on that whether it is accumulated inside the cell or it is on the cell surface. For example, unicellular microorganisms like magnetotactic bacteria synthesize magnetite nanoparticles in intracellular (Lovley *et al*. 1987). Extracellular synthesis is also reported (Bai *et al*. 2009) synthesis of CdS nanoparticles by the ecologically and environmentally important microorganism *Rhodobacter sphairoides* at room temperature. CdS nanoparticles can be synthesized on bacterial cellulose nanofibres which act as an excellent matrix (Li *et al*. 2009). Bacterial cellulose has more surface area containing hydroxyl and ether group which acts as the binding site for Cd^{+2} ions and simultaneously synthesizes CdS nanoparticles (Chen *et al.* 2009). Among the eukaryotes, yeasts are more preferably exploited for the synthesis of semiconductor CdS nanoparticles. When Cd^{+2} ions are exposed to *Candida glabrata* intracellular CdS quantum dots were formed (Reese and Winge 1988; Dameron *et al.* 1989). The use of fungi in biosynthesis of CdS is potentially more applicable, as it secretes a lagre number of enzymes and is very easy to handle in the laboratory. Das *et al.* (2012) synthesized CdS nanoparticle on functionalized *Aspergillus versicoolor* mycelia which have the potential to remove cadmium ions from aqueous solution and thus helps in bioremediation.

Now a day's more emphasis has given to surface and interfaces roles in various research areas like nanotechnology and environmental technology etc. Template directed synthesis of nanoscale materials has found to have various potential applications in photo catalysis, molecular electronics, active electronic devices and solar energy conversion (Du *et al*. 2005; Hoffman *et al*. 1992). The utilization of template molecules in the nanomaterial fabrication increases the accessibility for catalytic reactions and are currently being explored in various systems like metal oxide, silica, aluminium hydroxide coated phospholipid tubules, cellulose, polymers, carbon nanotubes etc. Among these materials biological templates are exploited more to modulate the synthesis of large number of inorganic nanoparticles including semiconductors, metals and magnetic particles. The biological materials are more important as they less likely create environmental pollution and effect over the other materials. The specific properties include precise molecular recognition and they imparts a spatial organization after the growth of nanoparticles on the biological materials through specific binding affinities, nucleation and assembly (Das *et al*. 2012).

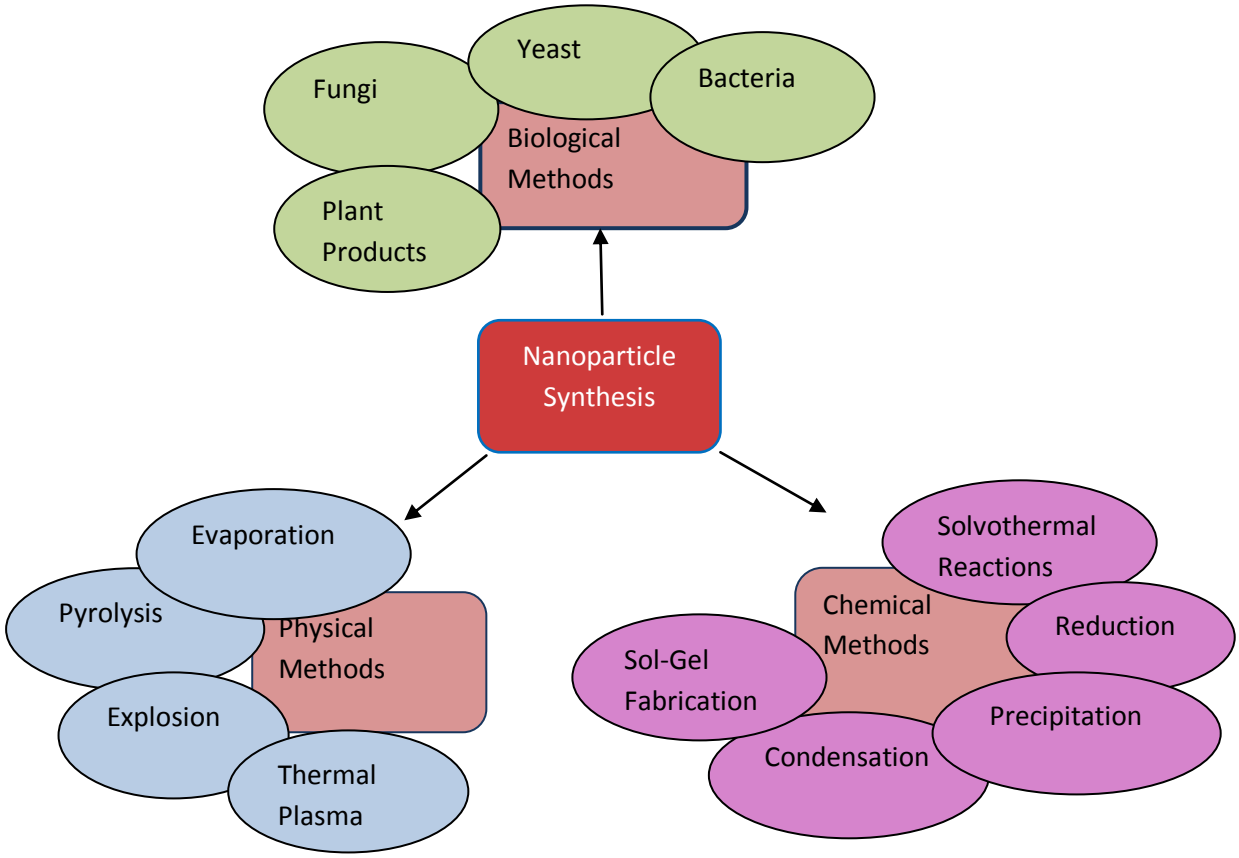


Fig. 2. Different methods for nanoparticles synthesis

This chemical modification creates preferential binding sites for nanoparticles to nucleate and organize in a better way. He *et al*. (2005) synthesized nano porous silver nanostructure using silver template. There is a controlled interaction between surface functional groups and the nanoparticles which have higher order hybrid assemblies. In current years among various nanoparticles, surface directed synthesis of Cadmium Sulphide nanoparticles has gained much more importance due to its various important properties. Biologically active multitasking bacteria apart from being a preferred choice for nanoparticle synthesis also have a very important role in remediation of contaminant from environment. Microbial based remediation is one aspect of bioremediation, where different adaptive processes of microorganisms with respect to contamination have been exploited for the remediation of environment. In other words "bioremediation is the use of microorganism's metabolism in the removal of environmental pollutants like heavy metals, pesticides and hydrocarbons etc." Bioremediation can be done either by treating the contaminants at the site or by treating elsewhere not at the site. Heavy metals are naturally occurring metals and metalloids in the earth crust which have

density greater than 4000kg m^{-3} four times greater than density of water (Garbarino *et al*. 1995). Some of the heavy metals are micronutrients to living organism but they are toxic if present in higher concentration. The toxic heavy metals are cadmium, lead, arsenic, mercury etc. which reacts with biomolecules to form stable bio-toxic compounds which are very difficult to dissociate (Duruibe *et al*. 2007). Cadmium is one of the toxic heavy metal found in the effluents of industries. Cadmium ions are not biodegradable and they accumulate in living organisms at a higher concentration and cause various health hazards. There are various techniques which are most commonly used to remove the heavy metals like cadmium from water bodies are ultrafiltration, lime precipitation, ion exchange and reverse osmosis, electrochemical treatment, oxidation-reduction (Das *et al*. 2012). But the use of these techniques have certain limitations like incomplete precipitation, high operating cost and generation of a large amount of metal bearing toxic sludge (Das *et al*. 2012). Bioremediation which involves the use of microorganisms and their products for detoxification and degradation of environmental pollutants like heavy metals has received increasing attention in the recent time. Growing of metal resistant cells at the site of contamination can ensure better removal trough combination of various processes like biosorption, bioprecipitation and continuous uptake of metals after physical adsorption. Marine environments have extreme and most dynamic environmental conditions that are completely different from the terrestrial environment. The microorganisms which thrive in these extreme and dynamic environments must have special metabolic pathways and mechanisms in order to survive in these extreme environments. So, marine microorganisms have better capability of producing more bioactive compounds to adapt them to that environment. Extracellular polymeric Substances (EPS) are found in the surrounding as the outer most structures of both prokaryotic and eukaryotic bacterial cells. They may be associated closely with the bacterial cell as capsule or may be remain unattached to the cell and secreted as slime. EPS are high molecular weight polymers of long chain sugar residues secreted by bacteria into surrounding. Along with polysaccharides it also contains proteins and nucleic acids that impart a variable molecular mass and properties. Microbial EPS have many functions like forming biofilm, protecting parasitic organisms from phagocytosis, defending microorganisms from predation and desiccation, storing nutrients, serving as surface adhesions, stabilizing enzymes etc (Weiner 1997). EPS also have vast industrial applications like emulsification, gel formation, film formation, absorption, anticancer treatment etc (Lee *et al*. 2003). The microbial EPSs have more advantage over plant and micro algal EPS due to their unique physical and chemical properties and novel functions. Bacterial

EPS contains charged functional groups that have specific adsorptive and adhesive properties and these charged groups serve as binding sites for many other charged groups including metal ions (Decho 1990). This metal binding or adsorption capacity of EPS led it to exploit in the bioremediation of different heavy metals like nickel, cadmium etc. But lack of affinity of EPS towards binding of metal requires a long time to reach equilibrium. This results in need to functionalize the EPS with some functional group that have a better binding capacity than the EPS towards metal ions. Generally the functionalization is done by amines, polyethylamine, crown ethers, sulphur bearing groups like thiols, dithiophosphates, xanthates. Among various sulphur containing groups' xanthate functionalization is preferred because of their easy preparation procedure, low solubility product and high stability constant of the metal complexes formed (Das *et al*. 2012). These microbial products along with nanoparticles could produce better yield in remediation of toxic metals then using them alone. This work is based on the above mention combinational approach of extracellular polymeric substances along with nanoparticles for removal of cadmium ions from its aqueous solution.

Review of Literature

In group II-VI semiconductor, Cadmium sulphide nanoparticles have generated much interest due to its size dependent properties. Bulk CdS has hexagonal wurtzite -type structure (Villars and Calvert 1985) melting point 1600 ^{o}C (Goldstein *et al.* 1992) and band gap energy which is 2.42 eV at room temperature and pressure. Due to size dependent properties, the melting point of 2.5 nm CdS crystallites was observed as low as ~ 400 ^{0}C and band gap energy of 0.7 nm CdS crystallites was found to be 3.85 eV (Banerjee *et al*. 2000). Very high pressure changes the phase of CdS nanoparticles from hexagonal wurtzite to rock salt cubic phase (Chen *et al.*1997). Various morphologies of CdS nanocrystals were so far reported in that flakes, spheres, dendrites, nanowires, nanorods, triangular, hexagonal and sea urchin like shape (Li *et al*. 1998; Qingqing *et al.* 2005; Gao *et al.* 2006; Zhao *et al*. 2006; Cheng *et al*. 2006) were dominant. Three different morphologies layers, hexagonal pore arrays and hollow spheres from H_2S and Cd^{+2} by lyotropic liquid crystal template were reported by Braun *et al.* (1999), however Chen *et al*. (2006) has synthesised unique nanometer dimension hollow spheres of CdS nanoparticles. Hollow spheres of CdS nanocrystals can also be synthesized in submicrometer range from the reaction of CS_2, $CdCl_2$ and ethylene diamine (Jiang *et al.* 2004) and by using the micelle formed by mixed solution of double hydrophilic block copolymer and surfactant as a soft template (Song *et al*. 2003). CdS nanoparticles can be synthesized in different phases like in solid phase, in liquid phase and in gas phase which is mainly relies on

the properties of material (Singh *et al.* 2010). Hydrothermal or solvo thermal is also an useful method of preparation of low dimensional sulphide nanomaterials, synthesis of flower like CdS and spherical ZnS microcrystallite in aqueous solution were also reported by these techniques (Wang *et al.* 2012). Micro and submicron CdS can be synthesized by the reaction between $CdCl_2$ and $Na_2S_2O_3$ in aqueous solution at low temperature (Zhang *et al*. 2008), whereas CdS nanoparticles have been synthesized by precipitation method using $CdCl_2$, H_2S and NaOH.
Recently, CdS nanoparticle with varied properties has been synthesized by polymers carrying functional groups which can act as a stabilizer. Polyester chain with thiol group, dimethylformamide and tetrahydrofuran can be used in the synthesis of stable CdS nanoparticles (Carrot *et al.* 1998). Amino derivative polysaccharides (Amdex) can be employed in the synthesis of CdS nanoparticles and the resultant Amdex-CdS nanoparticles complex can be conjugated or activated by antibody (Sondi *et al.* 2000). Qi *et al.* (2001) has synthesized CdS nanoparticle by using double hydrophilic copolymers such as PEG-b-PEI as a stabilizer which consists of a binding PEI block and a solvating PEG block. Various stabilizing agent used in the synthesis of CdS nanoparticles are thiophenol, thioglycerol, PMMA and PVA (Banerjee *et al*. 2000). CdS nanoparticles can be synthesized by different chemical methods such as, by using $CdCl_2$ with Na_2S with CTAB (Cetyl trimethyl ammonium bromide) as a surfactant (Singh *et al.* 2010), or methanol as the capping agent at room temperature (Mercy *et al.* 2013). CdS quantum dots (size smaller than 30nm) can be synthesized by using a chemical bath technique in which PVA (Poly Vinyl Alcohol) is used as a capping agent. Stable CdS nanoparticles with varied properties have been synthesized by a chemical reaction of $CdSO_4$ with $Na_2S_2O_3$ where thio-glycerol acted as the capping agent (Singh and Chauhan 2009). Prabhu and Khadar (2005) synthesized CdS nanoparticle from cadmium salt and sodium sulphide and triethanolamine as capping agent. Khan *et al*. (2011) has reported synthesis of CdS nanoparticles by a simple chemical reaction by using Cd $(NO_3)_2$ and Na_2S. Bansal *et al.* (2012) has synthesized CdS nanoparticles from Cd $(NO_3)_2$ and Na_2S using glucose as a capping agent. In all the methods, capping agent is used to maintain the proper dimension of synthesized particles in nanometer range and prevents the aggregation of nanoparticles. Surfactants, dendrimers and amphiphilic block copolymers have also been used for the preparation of CdS nanoparticles (Fasol 1998).

Apart from chemical synthesis other famous route of CdS nanoparticles synthesis is biosynthesis of CdS nanoparticles, which can be carried out by using microorganisms, fungi and plants. The microbes involves in synthesis should grab the Cd^{+2} ions from their metal

solution and accumulate them in a reduced state by different enzymes and metabolites secreted by their activity. Among the eukaryotic organisms, yeasts are the most explored bodies for synthesis of semiconductor nanoparticles. Intracellular CdS nanoparticles can be synthesized by the yeast *Schizosaccharomyces pombe* (Kowshik *et al.* 2002). When Cd^{+2} ions are exposed to *Candida glabrata,* intracellular CdS quantum dots were formed (Reese and Winge 1988; Dameron *et al.* 1989). In the presence of cadmium the synthesis of surfactin phytochelatin is activated. The phytochelatin contain cysteine which binds with cadmium and forms Cd-phytochelatin complexes which are then transported into vacuoles. The complex is then degraded and nanoparticles are formed. The use of fungi is recently added to the list of microorganisms for the synthesis of nanoparticles. They are potentially known to secrete large amounts of enzymes and they are very simple to handle in the laboratory. Fungi can reduce the metal ions to nanosized particles by two mechanisms extracellular and intracellular. The extracellular synthesis mechanism involves an enzyme NADPH dependent nitrate reductase which is secreted into the medium. This enzyme reduces the metal ions into nano level and converts NADPH to $NADP^{+}$. The other mechanism is intracellular in which the cell wall and the sugar present in the cell wall plays important role. There may be some enzymes in the cell wall or cytoplasmic membrane which can reduce the metal ions into nanoparticles. *Coriolus versicolor* has the ability to reduce cadmium ions to nanopartcles which are very stable without the addition of stabilizer. This fungus secretes an enzyme containing SH group which synthesizes nanoparticle (Das *et al.* 2012). *Fusarium oxysporum* secretes an extracellular enzyme which can mediate the extracellular synthesis of CdS nanoparticles (Lang *et al*. 2007). There are very less report regarding the alga- mediated synthesis of nanoparticles. *Phaeodactylum tricornutum*, a marine phytoplanktonic alga produces phytochelatin coated CdS nanocrystal in response to Cadmium in the medium (Scarano and Morelli 2003). In comparison to fungus and alga, bacteria are more diverse and adaptive organisms with easy availability. Bacteria are the highly exploited organisms in the synthesis of cadmium sulphide nanoparicles. *Clostridium thermoaceticum* can precipitate CdS nanoparticle on the cell surface and the medium from cadmium chloride ($CdCl_2$) in the presence of cysteine hydrochloride in the growth medium (Cunningham and Lundie 1993). In this process probably the cysteine act as a source of sulphide which combines with the cadmium to form CdS nanoparticle. *Klebsiella aerogenes*, when exposed to growth medium containing Cd^{+2} ions, 20-200 nm size CdS nanoparticles on its cell surface. The composition of buffer of the growth medium plays an important role in the synthesis CdS crystallites. When *Escherichia coli* is incubated with $CdCl_2$

and Na_2S solutions, intracellular CdS nanocrystals are formed (Sweeney *et al.* 2004). Micro and nano-fibre bacterial cellulose can provide an extensive surface area for cadmium ion adsorption, with more surface hydroxyl and ether group compared to other cellulose bacterial cellulose are better for CdS nanoparticles synthesis (Chen *et al.* 2009). Li *et al.* (2009) has reported synthesis of CdS nanocrystals on bacterial cellulose nanofibres. Bai et al. (2009) has synthesized CdS nanopartcles by exploiting one of the ecologically and environmentally important microorganism *Rhodobacter sphaeroides* and also reported later synthesis of CdS nanoparticle by a photosynthetic, purple non-sulphur bacteria *Rhodopseudomonas palustris* at room temperature. The CdS nanoparticles are first formed intracellularly and then transported to extracellular medium which can be separated easily. The culture supernatant of bacterial isolates *E.coli* ATCC 8739, *B.subtilis* ACTT 6633, *L.acidophilus* DSMZ 20079T can be used as a putative candidate for CdS nanoparticle synthesis (El-Shanshoury *et al*. 2012). The culture supernatant may contain some extracellularly secreted metabolites that produce CdS nanoparticles. *Bacillus amiloliquifaciens* produces surfactin which helps in stabilization of CdS nanoparticle (Singh *et al.* 2011).

Heavy metals are group of metal and metalloids, which are the natural component of earth crust. The most toxic forms of these metals are Cd^{+2}, Pb^{+2}, Hg^{+2}, Ag^{+} and As^{+3}. In environment the occurrence of heavy metals are generally more than the organic pollutant such as pesticides and hydrocarbons. Cadmium is one of the heavy metals found in the effluents which are discharged from industries involved in metallurgical alloying, metal plating, ceramics, mining and other industrial operations (Hutton *et al.* 1987). The various physicochemical technologies such as oxidation-reduction, electrochemical treatment, membrane separation and ion exchange are found to be inadequate and very expensive. So, bioremediation which involves the exploitation of microbes in degradation and detoxification of environmental contaminants have received much more attention in the recent times (Gadd 2000; Malik 2004; Farhadian *et al.* 2008). There will be better removal through combination of biosorption, bioprecipitation and physical adsorption when metal resistance cells are grown in the medium (Sprocati *et al.* 2006; Yi *et al*. 2007).

Cadmium sulphide is an extremely insoluble and stable, so sulphide producing microorganisms can be used at contamination site to detoxify the heavy metals (Czupyrna *et al*. 1989). Different aerobic bacteria and sulfate-reducing bacteria such as *Salmonella typhimurium, Pseudomonas aeruginosa* and *Treponema denticola* have the ability to produce hydrogen sulphide and precipitate cadmium (White and Gadd 1996; Wang *et al*. 1997; Bang *et al*. 2000).

Nowadays dead algae were successfully utilized in the adsorption of heavy metals (Leusch *et al*. 1995; Holan *et al*. 1998). The biomass of *Sargassum sp*. (Cruz *et al*. 2004) and *Chlamydomonas reinhardtii* (Xue *et al*. 1988) were shown to be particularly effective in the bioremediation of Cd. *Spirullina platensis* biomass can be potentially applied in the biosorption of cadmium (Solisio *et al*. 2008). Micro organisms alone not effective always for remediation of heavy metals, their products such as extracellular polymeric substances (EPS) can also be used for better results.

Bacterial extracellular polymeric substances are complex mixture of macro molecular poly electrolytes proteins, carbohydrates and nucleic acids which gives a unique molecular structure and properties. Generally, the EPS matrix is 0.2 to 1.0 μm thick but in some bacterial species the thickness of the EPS layer does not exceed values between 10 to 30 nm (Sleytr 1997). Polysaccharides and proteins are the best investigated substances of EPS and the presence of polypeptides in the EPS matrix is the characteristic of a few Gram +ve bacteria (Sutherland 2001; Sleytr 1997). The structure of polysaccharides varies according to the functional group present in the polysaccharides. Most heteropolysaccharides contains succinate, pyruvate and formate as the substituent (Sutherland 2001). The protein components of EPS are of molecular masses 10 to 50 kDa and are constitute about 40-60% hydrophobic amino acids. *Sulfolobus acidocalcidarius* synthesizes extracellular proteins which are mainly composed of amino acids with hydroxyl group. The EPS is either closely associated with the cell in the form of capsule or may be secreted as slime which are not attached to the cell. The EPS is thought to serve many functions like formation and maintenance of micro colony, promotion of initial attachment of cells onto solid surface, enhances the resistance of biofilm to external stress, helps in capture of nutrients and as a disinfectant (Dunne 2002; Pontefract 1991). The EPS production increased under the condition where the growth phase was extended by high glucose content. Low nitrogen content in the environment also influences the production of EPS (Fleming and Wing Ender 2001). The presence of high amount of nitrogen induces the synthesis of extracellular proteins (Sleytr 1997). When *Pseudomona* and *Rhodococcus* species were incubated in medium containing high ammonium salts, the biosynthesis of exogenous protein increased many folds. *Bacillus subtilis* known to synthesize extracellular polymeric substances like cellulose (Hestrin *et al.* 2004). In *Pseudomanas aeruginosa*, self-produced EPS are the signal for the growth of biofilm. Charged functional groups of high molecular weight polymers of bacterial EPS are reason for specific adsorptive and adhesive properties (Bhaskar *et al*. 2006). Due to presence of these charged groups, they

serve as binding sites for many other charged groups including metal ions (Decho 1990). The metal binding capacity of EPS is well studied and his metal binding or adsorption capacity of EPS led it to exploit in the bioremediation of different heavy metals like nickel, cadmium etc (Brown & Lester 1982; Loaëc *et al.* 1997; Dong *et al*. 2000). EPS can form multiple complexes with metals and have greater binding sites for metals than any other substances (Quigley *et al.* 2002). EPS helps in the immobilization of metal in the environment and makes it available for biological systems (Selck *et al.* 1999). When EPS producing organisms added into the feeds, they will ingest the toxic metals and other organic substances adsorbed on to the EPS thereby helping in the bioaccumulation of these metals and organic substance in tissues of animals (Harvey and Luoma 1985; Decho and Lopez 1993).

Adsorption is a recently developed technique for efficient removal of metal ions from aqueous solution in which the rate of adsorption is directly related to the quality of adsorbent. The combination of nanotechnology and separation technique has demonstrated high adsorption efficiency due to high surface area to volume ratio of nanoparticles (Gupta and Nayak 2012). Feng *et al.* (1992) and later Liang *et al.* (2009) has used orange peel for synthesis of Fe_3O_4 , Gupta and Nayak (2012) has further carried out the synthesis of novel nano-adsorbent by surface modification of Fe_3O_4 nanoparticle with orange peel powder for better adsorption and removal of metal ions. Orange peel is a non-toxic, low cost biosorbent of cellulose, hemicelluloses and pectin component containing hydroxyl and carboxyl groups. Jawor and Hoek (2010) have studied the relative ability of four nanoscale material poly (acrylic) acid, alginic acid, PAMAM dendrimers and Linde type A zeolite to bind with cadmium ions and to be subsequently removed from water by ultrafiltration. Surface functionalization of adsorbent material with some functional group has been believed to have greater affinity towards metal ions. Das *et al.* (2012) synthesized CdS nanoparticle on xanthate functionalized fungal mycelia of *Aspergillus versicolor* and used them in the removal of cadmium ions from its aqueous solution.

Objectives

1. Production and characterization of EPS from marine bacteria
2. Surface functionalization of pristine bacterial EPS
3. Synthesis and characterization of CdS nanoparticle from pristine EPS and glucose.
4. Synthesis and characterization of Cd based nanoparticles with surface functionalized bacterial EPS
5. Removal of cadmium from aqueous solution with EPS and CdS nanoparticles based adsorbent

Materials and Methods

Production and characterization of EPS from Marine Bacteria

Marine bacterial strains were isolated from different coastal regions of Odisha, out of which five EPS producing bacterial isolates were screened and taken for further experimental study. Each isolate was cultured aerobically for 24 hr in Luria Bertani broth at 37°C by shaking at 120 rpm. It was then centrifuged at 6900 rpm for 15 min and the cell pellet was again inoculated in Minimal Davis Broth supplemented with filter sterile 1% glucose and 20 mM $CaCl_2$ solution and incubated at 37°C and shaking condition. The cells were grown up to stationary phase and the cultures were transferred into 50 ml falcon tubes and centrifuged at 6900 rpm for 30 min at 4°C to separate the cell pellet. The supernatant was collected and equal volume of chilled ethanol (95%) was added slowly along the side wall of falcon tubes and incubated overnight at 4°C for precipitation of EPS. After overnight incubation it was then centrifuged at 6900 rpm at 4°C to separate the EPS. After centrifugation the pellet was collected and dried by desiccator. Among the five isolates of marine bacteria, better EPS production was observed in JP-11 which has been characterised as *Pseudomonas aeruginosa* by 16S rRNA gene sequencing and has been submitted to NCBI GenBank with the accession number KC771235, which we used in our future studies.

Characterization of EPS

The total carbohydrate content of EPS in the isolate JP-11 was estimated by phenol sulphuric acid method proposed by Dubois *et al*. (1956). The total amount of protein present in the isolate *P. aeruginosa* JP-11 EPS was estimated by Bradford method (1976).

X-Ray Diffraction study was performed by using Rigaku Miniflex X ray diffractometer, Japan. XRD was used to determine the lattice parameter, crystallite size and phase

identification. The dried EPS was scanned in the range 20^{o}-60^{o} with scanning rate 10^{o} to observe the characteristic peak for different functional group present in EPS.

Surface Functionalization of Pristine Bacterial EPS

50 mg of EPS extracted from *P. aeruginosa* JP-11 was taken in a 15 ml falcon tube. 750 µl of 4M NaOH was added to the EPS. It was then incubated for 2 hr at 120 rpm and 30^{o}C. After 2 hr of incubation 50 µl of carbon disulphide (CS_2) was added. It was again incubated for 3 hr at 120 rpm. Then the pellet was allowed to settle for 1 hr and the supernatant was decanted. The residue was washed thoroughly with double distilled water to make it alkali free and finally dried by washing with acetone. The dried residue was the stored under refrigerator condition until use.

Synthesis of Cadmium sulphide (CdS) nanoparticles from pristine bacterial EPS and glucose

Synthesis Using Glucose

Cadmium nitrate (Cd $(NO_3)_2$ and Sodium sulphide (Na_2S) were used for the synthesis of CdS nanoparticles and glucose was used as a capping agent. Different concentrations (0.1M, 0.01M, and 0.001M) of cadmium nitrate solutions were prepared and equal concentration of sodium sulphide (Na_2S) solution was added drop wise continuous stirring with a magnetic stirrer at 380rpm and 50^{o}C. The colour of solution changes to pale yellow. Then glucose (in mg) was added to the solution. The solution was allowed to stir for 20 hr. After 20 hr of stirring the pale yellow solution was transferred into 1.5mL eppendorf tubes and centrifuged at 12000 rpm at 25^{o}C for half an hour. The supernatant was decanted and pellet was collected. The pellet was washed 3 times with ethanol. The UV-Visible spectrophotometer reading of the pellet with ethanol was taken. The pellet was dried using desiccator. The table describing the different concentration of Cd $(NO_3)_2$, Na_2S and glucose is presented below (Table 1).

Table. 1. Different concentration of Cd $(NO_3)_2$, Na_2S and glucose used for CdS nanoparticles synthesis

Conc. of $Cd(NO_3)_2$ solution	**Conc. Of Na_2S solution**	**Weight of Glucose**
0.1M	0.1M	1mg
0.1M	0.1M	2mg
0.01M	0.01M	1mg
0.01M	0.01M	2mg
0.001M	0.001M	1mg
0.001M	0.001M	2mg

Biological synthesis using pristine EPS

The nanoparticle was synthesized at different concentrations (0.1M, 0.01M, 0.02M and 0.001M) of $Cd(NO_3)_2$ and Na_2S solution using the same procedure as chemical synthesis using glucose. The colour of the solution changed to pale yellow. After 20 hr of stirring the solution was transferred into 1.5mL eppendorf tubes and centrifuged at 12000rpm and 25^oC for 30 min. The supernatant was decanted and pellet was collected and washed 3 times with 95% ethanol. Then was scanned using UV-Visible spectrophotometer to find the characteristic peak for CdS nanoparticles. The pellet was dried using desiccator. The table describing the different concentration of Cd $(NO_3)_2$, Na_2S and EPS is presented below (Table 2).

Table. 2. Different concentration of Cd $(NO_3)_2$, Na_2S and EPS used for CdS nanoparticles synthesis

Conc. of $Cd(NO_3)_2$ solution	**Conc. Of Na_2S solution**	**Weight of EPS**
0.1M	0.1M	1mg
0.1M	0.1M	2mg
0.01M	0.01M	1mg
0.01M	0.01M	2mg
0.02M	0.02M	1mg
0.02M	0.02M	2mg
0.001M	0.001M	1mg
0.001M	0.001M	2mg

Characterization of synthesized of nanoparticles from chemical and biological sources

The absorption spectra of nanoparticles synthesized by various methods were recorded by scanning the samples in the range 200 to 700nm by using Double Beam Scanning UV Visible spectrophotometer.

X-Ray Diffraction pattern was recorded by using Rigaku Miniflex X-ray diffractometer, Japan. XRD data were used to determine crystalline nature and phase identification. The samples were scanned in the range 20^{o}-60^{o} with scanning rate 10^{o} to observe the characteristic peak at angle of diffraction of 2θ degree.

The synthesis of CdS nanoparticles on bacterial EPS was characterized by Nova NanoSEM field emission scanning electron microscope equipped with an energy dispersive X-ray spectrometer (EDX). Before analysis the samples are coated with gold.

The ATR-FTIR spectra of nanoparticles synthesized by various methods were taken to observe the characteristic peak of each of the functional group present in the sample. Before doing this the samples are dissolved in PBS and sonicated to get disperse suspension.

Synthesis and Characterization of CdS nanoparticles using Functionalized EPS

10 mL of 0.01M Cd $(NO_3)_2$ solution was taken in 15 mL falcon tube and 20 mg of functionalized EPS was added to it. The solution was stirred at 380 rpm and 50^{o}C for 20 hours. After 20 hr of stirring, the solution was transferred to 1.5mL eppendorf tubes and centrifuged at 12000 rpm and 25^{o}C for 30min. The pellet was collected and washed with 95% ethanol and then dried by desiccator.

The absorption spectrum of nanoparticles synthesized by functionalized EPS was recorded by scanning the samples in the range 200 to 700nm by using Double Beam Scanning UV-Visible spectrophotometer.

X-Ray Diffraction was performed by using Rigaku Miniflex X ray diffractometer, Japan. The sample was scanned in the range 20^{o}-60^{o} with scanning rate 10^{o} to observe the characteristic peak at angle of diffraction of 2θ degree.

Batch adsorption experiment for cadmium removal from aqueous solutions

Adsorption experiment were conducted in batch process in 15 ml falcon tubes to study the cadmium ion uptake capacity of different nanoparticles synthesized by using glucose, EPS and functionalized EPS. The optimum pH for adsorption was determined by suspending 4mg/mL functionalized EPS of isolate JP-11 in 15 ml falcon tubes containing 50 ppm cadmium ions of different pH 2.6, 4.6 and 6.6. Different volumes of 0.1M citric acid and 0.2M dibasic sodium phosphate was used to prepare different pH solution containing 50 ppm concentration

of cadmium. The optimum pH for maximum adsorption was found to be 4.6 from previous experiments. Citrate phosphate buffer (2.85 ml) was taken in 5 falcon tubes and 150 µl of 1000 ppm Cd $(NO_3)_2$ solutions was added to make the final concentration 50 ppm. 12 mg of total nanoparticles synthesized from glucose, EPS and functionalized EPS were taken respectively to remove the cadmium from aqueous solution. The falcon tubes were incubated at 120 rpm and 37°C for 24 hr. After 24 hr of incubation, the solution was centrifuged at 6900 rpm to separate the adsorbent from the supernatant. The optical density of the supernatant was recorded by UV-Visible spectrophotometer. From the standard curve of cadmium, the concentration of cadmium removed from the aqueous solution was calculated. The UV-Visible spectrophotometer reading was taken at 24 hr and 48 hr of incubation. After 48 hr of incubation the sample was centrifuged to separate the pellet. The pellet was dried by using desiccator.

Results

Characterization of EPS

With standard curve of carbohydrate (D-glucose) obtained by phenol sulphuric acid method, the maximum carbohydrate content of the EPS from the isolate *P. aeruginosa* JP-11 was 4919.18 µg/ml. With the standard curve of protein (BSA), the amount of protein content in the EPS was 2160.92 µg/ml.

The XRD result of bacterial EPS revealed many peaks at 20.76°, 23.26°, 29.09°, 30.44°, 31.17°, 33.88°, 41.28°, 50.03° (Fig. 3).

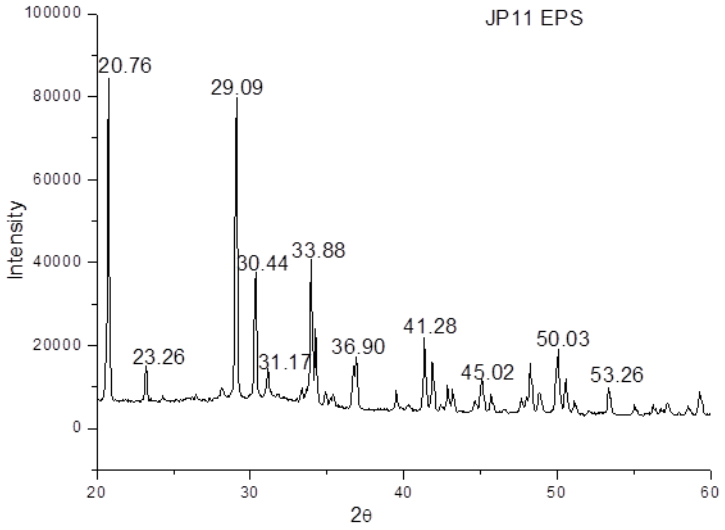


Fig. 3. The XRD pattern of pristine EPS from the isolate JP-11

Surface functionalization of the pristine bacterial EPS

The pristine bacterial EPS after treatment with the solution of NaOH was completely turbid white. After two hr of incubation the colour of the solution changed from white to light yellow. When CS_2 was added to the solution, the colour remained the same, but after 3 hr of incubation the colour changed to deep orange (Fig. 4).

(a) **(b)** **(c)**

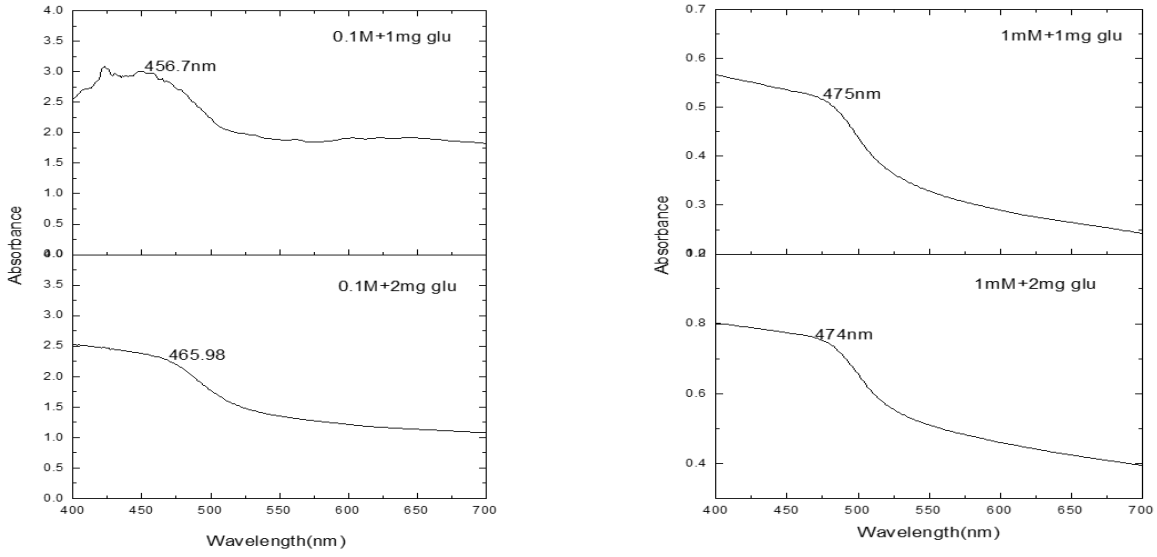
Fig. 4. Change in color of Pristine EPS **(a)** in NaOH, **(b)** after 2 hr incubation with NaOH **(c)** after 5 hr incubation with NaOH and CS_2

Characterization of CdS nanoparticles from EPS and glucose

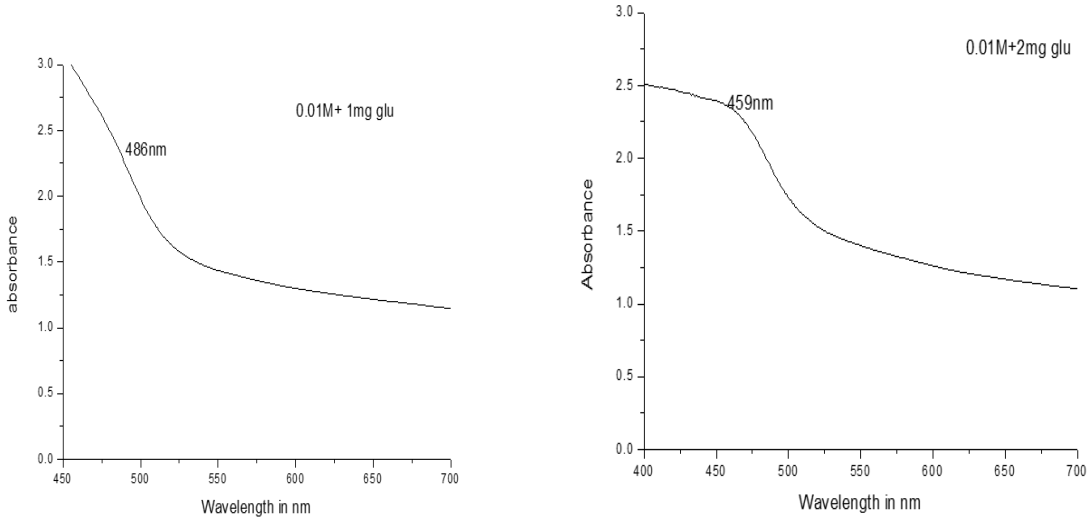
Addition of equal concentration of Na_2S solution drop wise and glucose to Cd $(NO_3)_2$ with continuous stirring, changed the colour of solution from white to pale yellow and then to orange yellow indicating the formation of CdS nanoparticle. The chemical synthesis of CdS nanoparticle using bacterial EPS has also shown the same colour changing pattern, confirming the synthesis of CdS nanoparticles. The pale orange colour nanoparticles synthesized by these methods were collected by centrifugation and washed two times with 95% ethanol and finally dried in a desiccator.

UV- Visible Spectra

Further characterization of CdS nanoparticle was confirmed by studying the optical properties. The yellow colour powder synthesized by using glucose and EPS were dispersed in ethanol and the UV-Vis spectra of dispersed solution was recorded by scanning in the range 200-700 nm (Fig. 5; Fig. 6). The UV-Vis spectra of the dispersed solution of the yellow colour powder synthesized using glucose and EPS showed absorption maxima between the wavelength 400-500 nm due to surface plasmon resonance band of the CdS nanoparticle. This confirms a blue shift from the bulk CdS which has absorption maxima of about 415 nm.

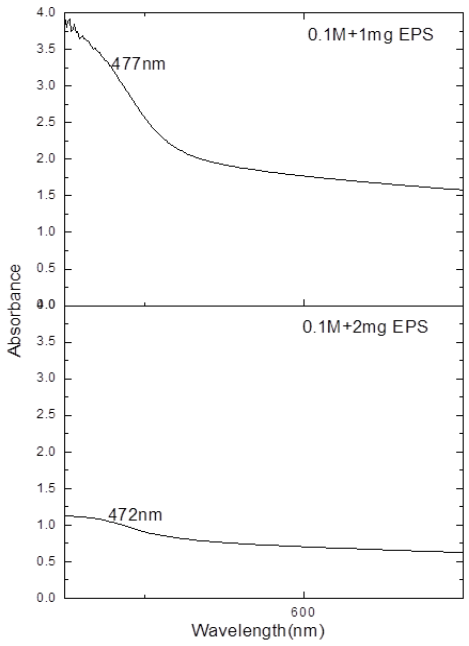


a) **b)**

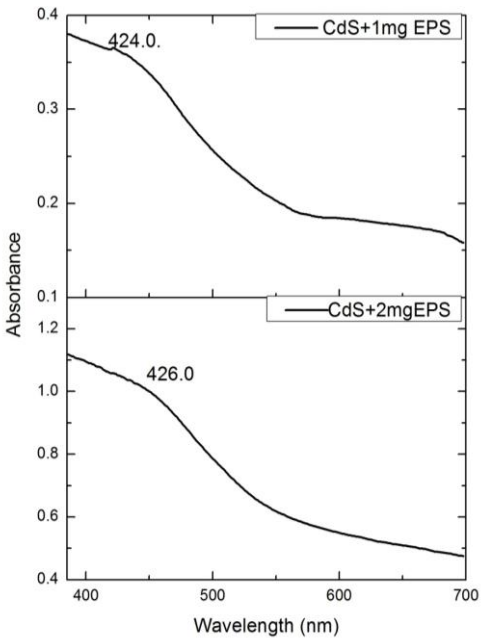


c) **d)**

Fig. 5. UV- Vis spectra of CdS nanoparticle synthesized by glucose, **a)** 0.1M $Cd(NO_3)_2$, 0.1M Na_2S (1mg and 2mg glucose), **b)** 1mM $Cd(NO_3)_2$, 1mm Na_2S (1mg and 2mg glucose), **c)** 0.01M $Cd(NO_3)_2$, 0.01M Na_2S (1mg glucose), **d)** 0.01M $Cd(NO_3)_2$, 0.01M Na_2S (2mg glucose)



a)



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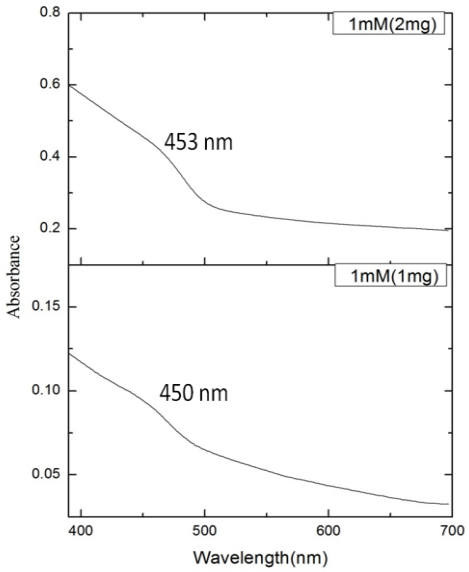
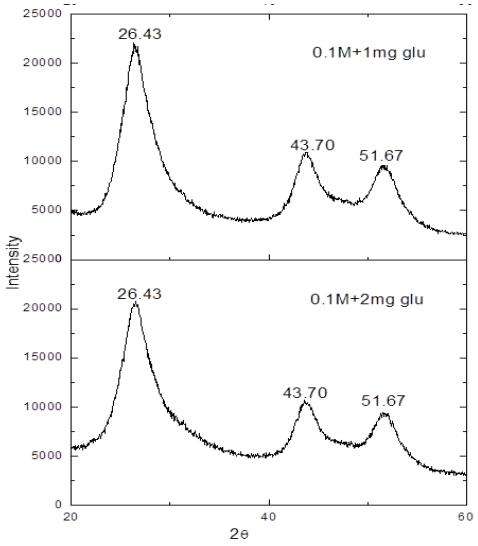


c)

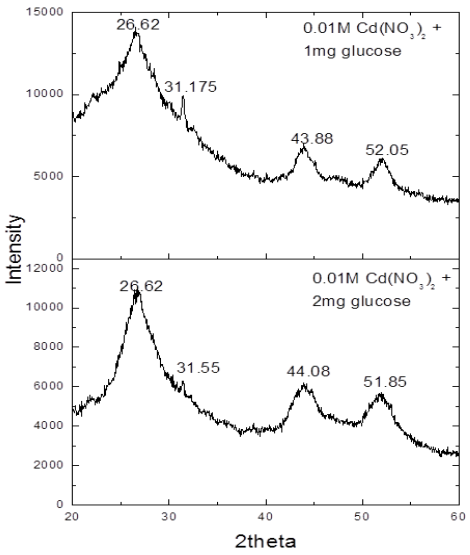
Fig. 6. UV-Vis spectra of CdS nanoparticle synthesized using EPS. **a)** 0.1M Cd $(NO_3)_2$, 0.1M Na_2S (1mg and 2mg EPS), **b)** 0.01M Cd $(NO_3)_2$, 0.01M Na_2S (1mg and 2mg EPS), **c)** 1mM Cd $(NO_3)_2$, 1mM Na_2S (1mg and 2mg EPS)

X-Ray Diffraction Pattern

The XRD patterns also confirmed the formation of CdS nanoparticles in different methods using glucose and EPS. The XRD pattern exhibited diffraction peaks at 26.4°, 43.7° and 51.6° corresponds to (111), (220), (311) planes of cubic phase CdS nanoparticles. The synthesis and crystalline nature was significantly influenced by the presence of capping agent. It was observed that the diffraction peak in presence of EPS is broader than the pure CdS (Fig. 7, Fig. 8). This result confirms that in presence of EPS the crystallite size of the CdS nanoparticle decreases to some extent. The CdS nanoparticles synthesized on functionalized EPS also shows peak at 26.48° which confirms the formation of CdS nanoparticles.



a)



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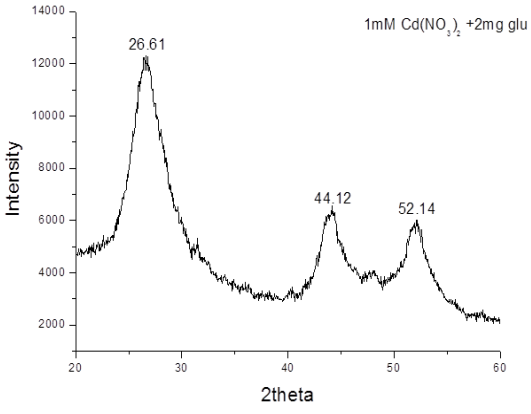
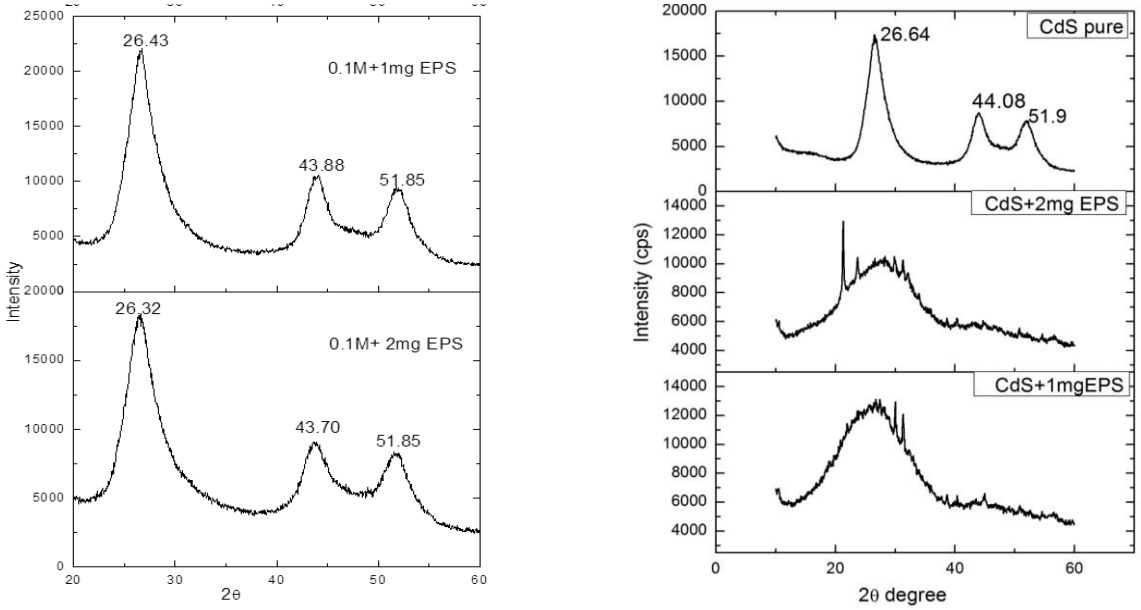


c)

Fig. 7. XRD Pattern of CdS nanoparticles synthesized by Glucose, **a)** 0.1M Cd $(NO_3)_2$, 0.1M Na_2S (1mg and glucose), **b)** 0.01M Cd $(NO_3)_2$, 0.01M Na_2S (1mg and 2mg glucose), **c)** 1mM Cd $(NO_3)_2$, 1mM Na_2S (2mg glucose)



a) **b)**

Fig. 8. XRD pattern of CdS nanoparticles synthesized by using EPS **a)** 0.1M Cd $(NO_3)_2$, 0.1M Na_2S (1mg and EPS), **b)** 0.01M Cd $(NO_3)_2$, 0.01M Na_2S (1mg and 2mg EPS)

ATR-FTIR:

According to spectroscopic data table, the downfield shift peak between 2300 cm^{-1} to 2600 cm^{-1} shows the presence of -SH functional group. The nanoparticles synthesized by using glucose and EPS showed peak in the range 2330-2360cm^{-1}, which confirms the presence of -SH group. The ATR-FTIR spectra thus confirmed the presence of sulphur group which can bind with cadmium and forms CdS nanoparticle (Fig. 9). The absorption band of CdS stretch was not observed in the current scale of spectrum as it is appeared at around 250 cm^{-1}.

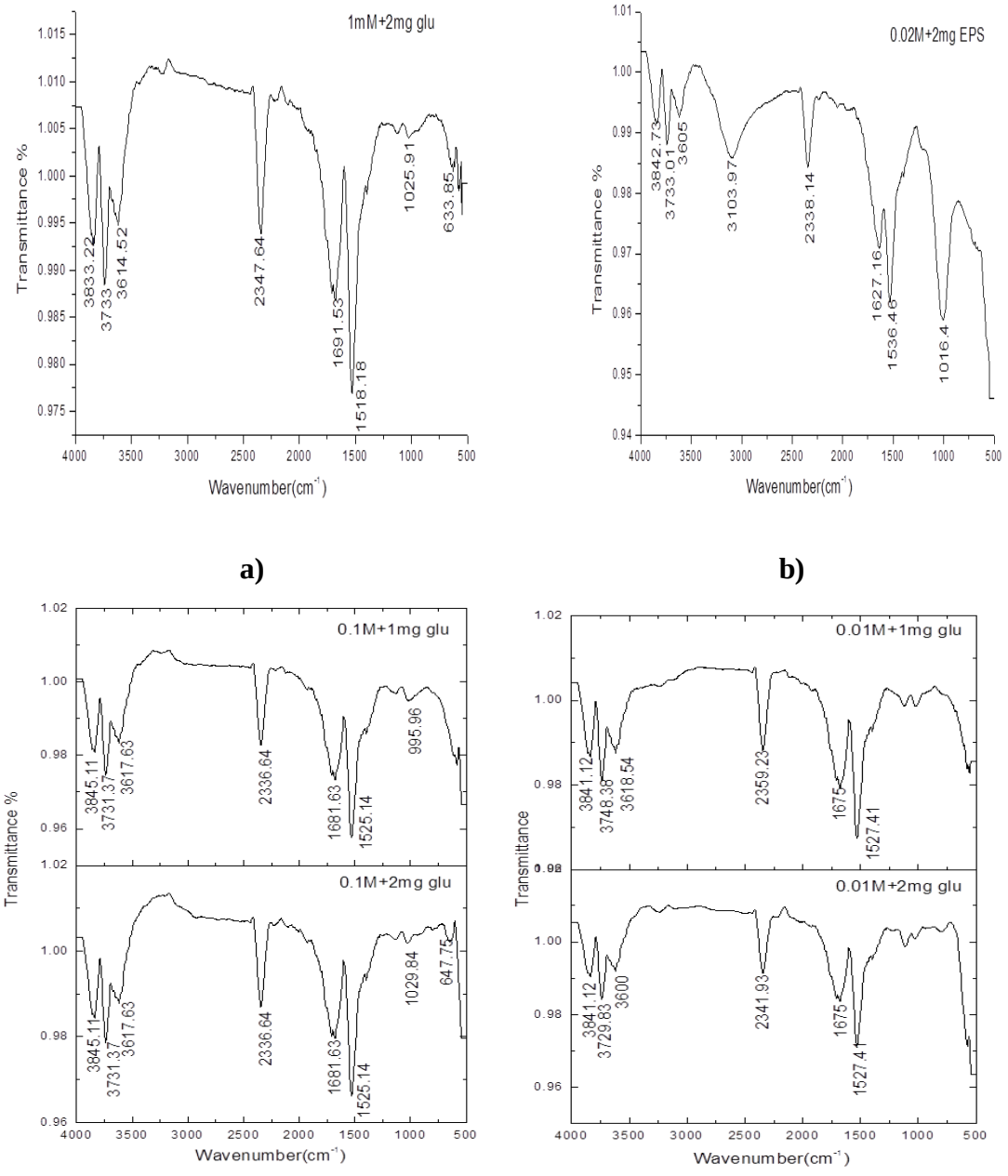
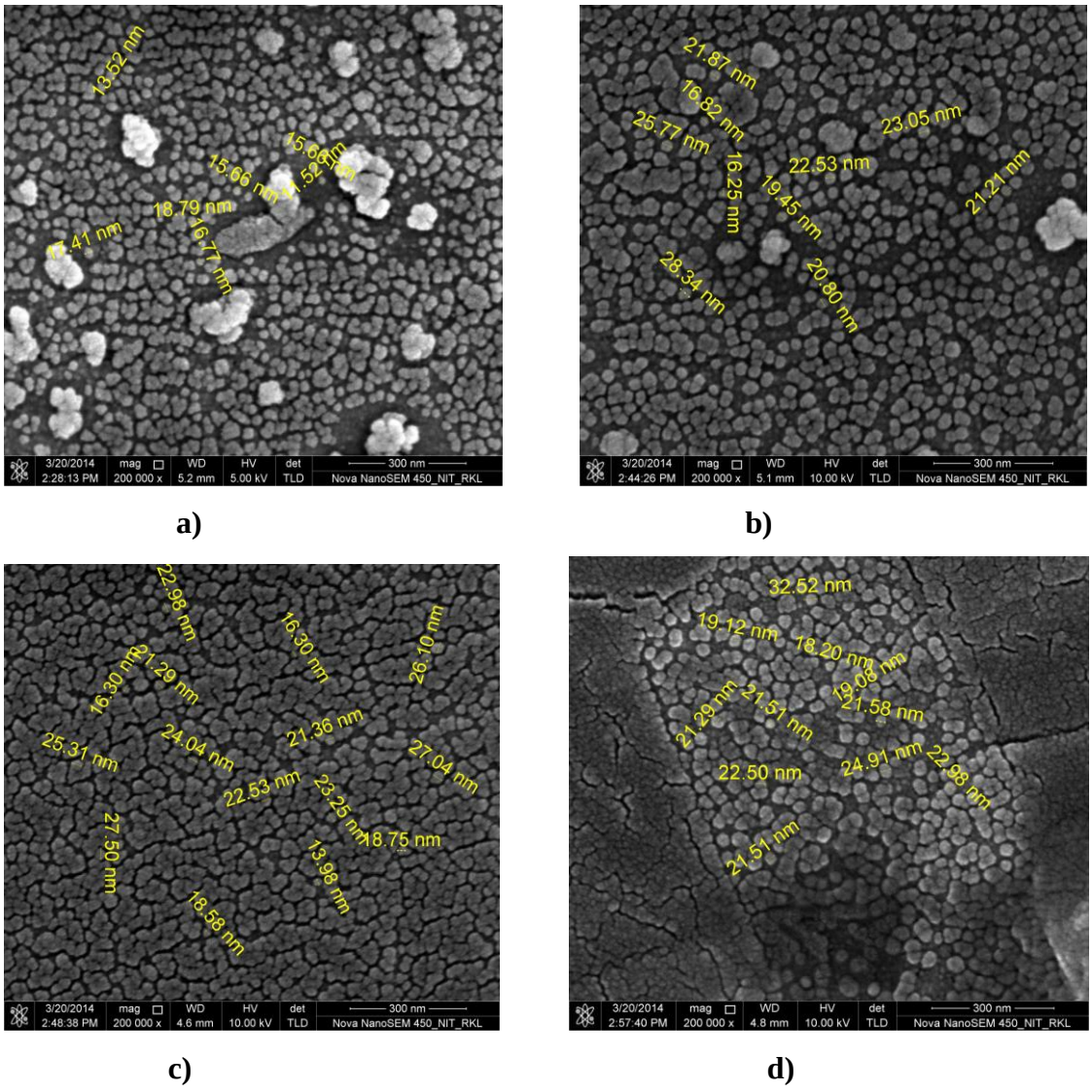


Fig. 9. ATR result of CdS nanoparticle by glucose and EPS, **(a)** 1mM $Cd(NO_3)_2$, 1mM Na_2S(2mg glucose), **(b)** 0.2M $Cd(NO_3)_2$, 0.2M Na_2S (2mg EPS), **(c)** 0.1M $Cd(NO_3)_2$, 0.1M Na_2S (1mg and 2mg glucose), **(d)** 0.01M $Cd(NO_3)_2$, 0.01M Na_2S (1mg and 2mg glucose).

FESEM characterization

FESEM images showed the surface morphology of nanoparticles synthesized by using pristine EPS and glucose. The average size of CdS naoparticle synthesized using glucose was found to be 17 nm and 20 nm when glucose concentration is 1 mg and 2 mg respectively. CdS

nanoparticle of size average size 22nm and 24nm were found when EPS is used instead of glucose (Fig. 10).



a) **b)**

c) **d)**

Fig. 10. FESEM image of CdS nanoparticle by glucose and EPS, **a)** 0.1M $Cd(NO_3)_2$, 0.1M Na_2S (1mg glucose), **b)** 0.1M $Cd(NO_3)_2$, 0.1M Na_2S (2mg glucose), **c)** 0.01M $Cd(NO_3)_2$, 0.01M Na_2S (1mg EPS), **d)** 0.01M $Cd(NO)_3$, 0.01M Na_2S (2mg EPS).

Characterization of CdS nanoparticles synthesized by Functionalized EPS

The UV- Vis spectra of Cadmium treated functionalized EPS give the absorption maxima at the wavelength at 451.78 nm which is the characteristic peak of CdS nanoparticle (Fig. 11).

The XRD graph of cadmium treated functionalized EPS was shown to give the peak at 26.48^o which corresponds to cubic phase of CdS nanoparticle (Fig. 12).

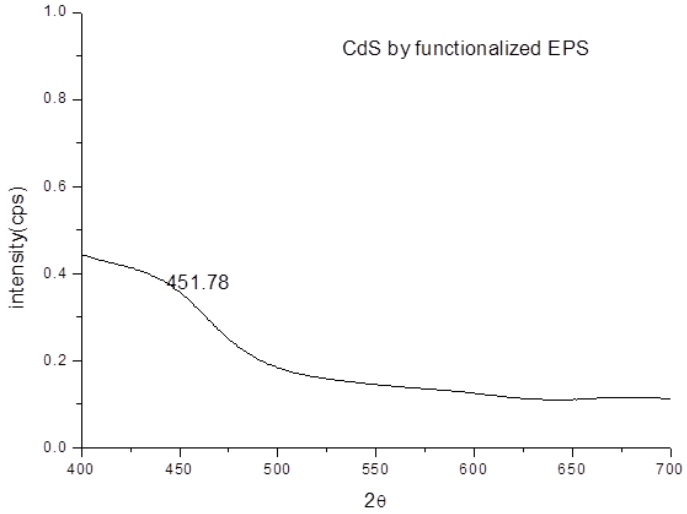


Fig. 11. UV- Vis Spectra of CdS nanoparticles formed by functionalized EPS

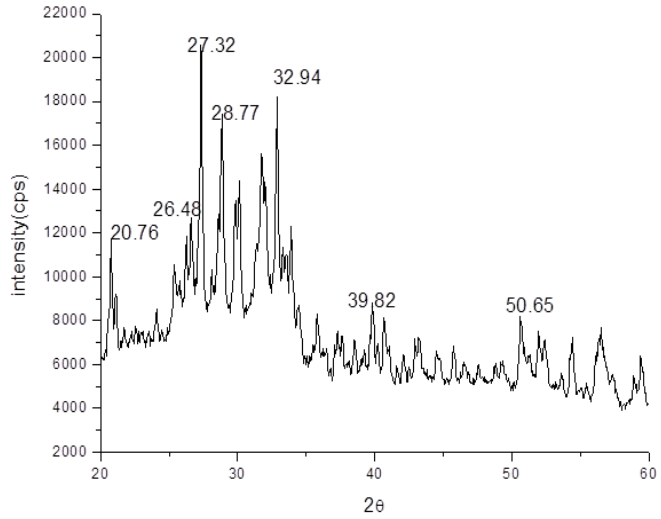


Fig. 12. XRD pattern of CdS nanoparticles on functionalized EPS

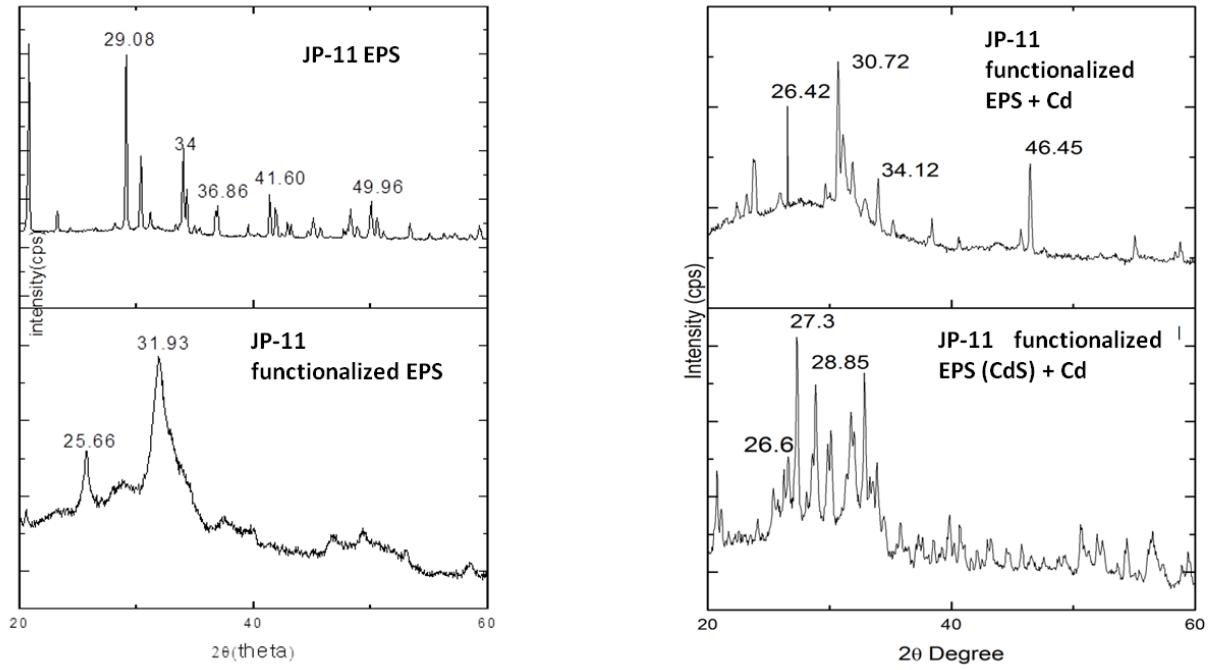
Batch Adsorption Experiment

The percentage of cadmium removal by the pristine EPS; functionalized EPS and CdS nanoparticles incorporated functionalized EPS at 24 hr and 48 hr were found to be 57.45%, 61.44%; 77.34%, 80% and 79.48%, 83% respectively. The rate of cadmium adsorption by the functionalized EPS of the isolate JP-11 was found to increase with incubation time. The optical density (OD) of the aqueous solution was measured after 24 and 48 hr of incubation, and it was observed that the OD has decreased with the incubation time (Table. 3).

Table 3: Percentage removal of cadmium from aqueous solution

Adsorbent	% removal of Cd (24 hr)	% removal of Cd (48 hr)
CdS nanoparticles synthesized by glucose	No adsorption	No adsorption
CdS nanoparticles synthesized by EPS	No adsorption	No adsorption
Pristine EPS	57.45	61.44
Functionalized EPS	77.34	80
CdS nanoparticles on functionalized EPS	79.48	83.42

The XRD spectra of pristine EPS and funtionalized EPS did not show any peak at around 26^{o} but when the surface functionalized EPS was treated with cadmium, the peak was observed at 26.42^{o} which is the characteristic peak of CdS nanoparticles. Moreover, the CdS nanoparticles incorporated funtionalized EPS when treated with Cd also showed peak at 26.6^{o} (Fig. 13). These results confirm that after adsorption of cadmium by surface functionalized EPS, it may have synthesized CdS nanoparticles on its surface.



a) **b)**

Fig. 13. XRD pattern of **a)** EPS, functionalized EPS, **b)** cadmium treated functionalized EPS, CdS nanoparticles synthesized on cadmium treated functionalized EPS of JP-11

Discussion

Increasing awareness among the people towards green chemistry and biological process has led to development of environmental-friendly approaches for the synthesis of nanomaterials. Previous experiments suggest that nanoparticles can be synthesized biologically by using plant products, fungi, yeast and bacteria. Based on previous reports, our study involved a comparison between CdS nanoparticles synthesis from chemical as well biological origin (EPS) and their ability in the removal of cadmium ions from aqueous solution. Moreover, marine bacteria are the potential candidates for many activities. They secrete extracellular polymeric substances (EPS) which have strong affinity towards metal ions and helps in their adsorption. But lack of affinity and inadequate uptake capacity of adsorbent requires a long time to reach equilibrium (Bayramoglu *et al*. 2002; Hall-Stoodley *et al*. 2004). So, surface functionalization of EPS with some functional group was studied to increase the uptake capacity of pristine bacterial EPS. In chemical synthesis, glucose is used as capping agent which prevents the aggregation of nanoparticles into bulk material and therefore helps in the synthesis of cadmium sulphide nanoparticles (Bansal *et al*. 2012). Previously, it has been

reported the usage of bacterial cellulose nanofibres for CdS nanoparticles synthesis (Li *et al*. 2008). In context to that, we have approached for the synthesis of CdS nanoparticles by using bacterial EPS as the capping agent. Bacterial EPS is composed of carbohydrate moieties which may act as capping agent for cadmium sulphide nanoparticles synthesis. The presence of various functional groups present in the EPS can be effectively harnessed for surface functionalization which can act as an adsorbent for removing metal ions. The functionalized EPS in which a chemical sulphur group is attached to its surface has a greater affinity towards Cd^{+2} ions and have greater potential of nanoparticle synthesis (Winter 1994). The UV-Visible spectra of CdS nanoparticles synthesized by chemical method gave the absorption peak in between 450-500 nm which shows blue shifts from cadmium bulk material. This result is in accordance with the previous study carried out by Liu *et al*. (2006). This blue shift can be attributed to transition from CdS bulk material to CdS nanoparticle in the presence of capping agent glucose. The bacterial EPS as the capping agent could also synthesize CdS nanoparticle. The UV-Vis spectra of synthesized CdS nanoparticle by using EPS as the capping agent showed characteristic absorption peak and blue shift that is similar to the nanoparticles synthesized by glucose. The UV-Vis spectrum of functionalized EPS treated with cadmium solution shows absorption peak at 451nm which demonstrated the characteristic peak of CdS nanoparticles.

The XRD pattern of chemically synthesized CdS nanoparticles shows absorption peaks at 26.4^{o}, 43.7^{o} and 51.6^{o} which corresponds to (111), (220), (311) planes of cubic phase CdS nanoparticles. This result was in concert to the previously reported XRD patterns of CdS nanoparticles (Li *et al*. 2009). The same absorption peaks were also observed when EPS was used for synthesis of CdS nanoparticles. In the presence of EPS the peak was quite broader than the CdS nanoparticles synthesized without it, which signifies the reduction in crystalline nature of the synthesized nanoparticles.

The ATR-FTIR results shows that there is no adsorption band for CdS nanoparticles in the current scale but they shows a downfield shifts of peak at 2330-2360 cm^{-1} which corresponds to -SH functional group. From previous studies also we have found that this sulphydryl group acts an important attachment group in the EPS (Rao 1992). The sulphur group act as a binding site for cadmium ions which after binding synthesizes CdS nanoparticles (Das *et al*. 2012). The proteins present in EPS either contain the sulphur group or any other charged group which after binding with cadmium synthesizes the CdS nanoparticles.

The FESEM results of the synthesised nanoparticles chemically and biologically showed the surface morphology and size of nanoparticles. The average size of CdS nanoparticles synthesized by chemical method using glucose was found to be 17-20 nm and while those synthesized by EPS was 22-24 nm. The images showed that the nanoparticles synthesized were homogenous with regular shape and uniform morphology.

The CdS nanoparticles synthesized by glucose and EPS as the capping agent, pristine EPS, functionalized EPS and nanoparticle incorporated functionalized EPS were tested for their cadmium adsorption capacity. Pristine EPS, functionalized EPS and the nanoparticles incorporated functionalized EPS were observed to remove 61%, 80% and 83% of cadmium from the aqueous solution respectively, whereas CdS nanoparticles alone (synthesized by glucose and EPS as the capping agent respectively) did not show significant adsorption capacity. The functionalized EPS was observed to have better adsorption capacity than the pristine EPS, as the sulphur group present in the functionalized EPS has greater affinity for cadmium. pH is an important factor in the adsorption of metal ions by changing the surface charge of both adsorbent and adsorbate. pH 5-6 was found to be the optimum pH for better adsorption of metal ions because at this pH, the net charge on cadmium is positive which corresponds to better adsorption (Das *et al*. 2012). However, in the presented work, it has been observed that the nanoparticles incorporated functionalized EPS has greater adsorption capacity than the functionalized EPS. This may be due to the fact that the nanoparticles provide enhanced surface area for adsorption of cadmium ions.

The XRD pattern of functionalized EPS after treatment with cadmium shows the characteristic peak that corresponds to the cubic phase of CdS nanoparticles (Bai *et al*. 2009). While, the pristine EPS treated with cadmium did not show any characteristic peak of CdS nanoparticles. The CdS nanoparticles incorporated functionalized EPS has the highest affinity for metal ion adsorption. The nanoparticles have a greater surface area to volume ratio, which provides a greater surface area for catalytic activity that confers to more cadmium ion adsorption (Feng *et al*. 2009). The functionalized EPS contains sulphur groups, so when added to aqueous solution containing cadmium ions, the sulphur group binds with the cadmium and forms CdS nanoparticles. Previous studies by Das *et al*. (2012) illustrated that the functionalized mycelia of *Aspergillus versicolor* could remove cadmium from its aqueous solution and at same time synthesize CdS nanoparticles on its surface. In perspective to fungal mycelia, present work has used functionalized bacterial EPS for synthesis of CdS nanoparticles

and removal of cadmium from its aqueous solution which could be a novel and most efficient technique for bioremediation of heavy metals.

Conclusion

The synthesis of nanoparticles using physical and chemical methods involves various chemical hazards and can cause environmental pollution, so biosynthesis of nanoparticles using microorganisms is an emerging approach in the field of nanotechnology. Extracellular polymeric substances (EPS) can serve as binding sites for various metal ions and also act as a capping agent in the synthesis of nanoparticles. Surface functionalization of EPS can enhance the adsorption of metal. Among the various functional groups sulphur is mostly used because of its high stability constant, low solubility products and easy preparation procedure. The sulphur group can easily bind with the cadmium ions in the aqueous medium and synthesizes CdS nanoparticles. The CdS nanoparticles incorporated into the functionalized EPS has a greater adsorption potential for metal ions than the functionalized and pristine EPS. So the presented work showcased a novel method for the synthesis of CdS nanoparticles using the bacterial EPS that also helps in removal of cadmium from aqueous solution. The EPS with CdS nanoparticles not only has better adsorption capacity but also has shown additional benefits like easy recovery, easy synthesis, and absence of secondary pollutant, cost-effectiveness and environmental-friendly. Thus, by further advancement of biotechnological and nanotechnological approaches, removal of different heavy metals from environment could be improved by enhanced adsorption and recovery.

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